A clinical, histopathological and laboratory study of 19 consecutive Italian paediatric patients with chilblain-like lesions: lights and shadows on the relationship with COVID-19 infection

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ABSTRACT

Background

Acral chilblain-like lesions are being increasingly reported during COVID-19 pandemic. However, only few patients proved positivity for SARS-CoV-2 infection. The relationship between this skin manifestation and COVID-19 infection has not been clarified yet.

Objective

To thoroughly characterize a prospective group of patients with chilblain-like lesions, and to investigate the possible relationship with SARS-CoV-2 infection.

Methods

Following informed consent, patients underwent: (i) clinical evaluation, (ii) RT-PCR and serology testing for SARS-CoV-2, (iii) digital videocapillaroscopy of finger- and toe-nailfolds, (iv) blood testing to screen for autoimmune diseases and coagulation anomalies, and (v) skin biopsy for histopathology, direct immunofluorescence, and, in selected cases, electron microscopy.

Results

Nineteen patients, all adolescents (mean age: 14 years), were recruited. 11/19 (58%) of them and/or their cohabitants reported flu-like symptoms one to two months prior to skin manifestation onset. Lesions were localized to toes and also heels and soles. Videocapillarcosopy showed pericapillary oedema, dilated and abnormal capillaries, and microhemorrhages both in finger and toe in the majority of patients. Major pathological findings included: epidermal basal layer vacuolation, papillary dermis oedema and erythrocyte extravasation, perivascular and perieccrine dermal lymphocytic infiltrate, and mucin deposition in the dermis and hypodermis; dermal vessel thrombi were observed in 2 cases. Blood exams were normal. Nasopharyngeal swab for SARS-CoV-2 and IgG serology for SARS-CoV-2 nucleocapsid protein were negative. Importantly, IgA

serology for S1 domain of SARS-CoV-2 spike protein was positive in 6 patients and borderline in 3.

Conclusions

Chilblain-like lesions during COVID-19 pandemic have specific epidemiologic, clinical, capillaroscopic and histopathological characteristics, which distinguish them from idiopathic perniosis. Though we could not formally prove SARS-CoV-2 infection in our patients, history data and the detection of anti-SARS-COV-2 IgA strongly suggest a relationship between skin lesions and COVID-19. Further investigations on the mechanisms of SARS-CoV-2 infection in children and pathogenesis of chilblain-like lesions are warranted.

INTRODUCTION

Chilblains, also called pernio, are an inflammatory skin manifestation characterized by erythema and swelling of the extremities, lasting more than 24 hours (1, 2). The disease presents following exposure to cold humid weather, usually during winter or early spring. It affects more frequently young and middle-aged women. Chilblains may be idiopathic or, less commonly, occur in association with an underlying autoimmune connective tissue disease, in particular lupus erythematosus, hematologic disorders, viral hepatitis, and malignancy. Chilblain lesions are edematous, erythematous to violaceous macules, papules, plaques, or nodules on the dorsal fingers or toes, and less frequently, on the nose, ears, and foot soles. Erosions or ulcerations may develop. Symptoms comprise burning, tenderness, or pruritus. Idiopathic chilblains usually run a benign course with spontaneous resolution in one to 3 weeks; however, annual recurrences are frequent.

COVID-19 pandemic is caused by a novel coronavirus, SARS-CoV-2, first isolated in Chinese patients with severe pneumonia and rapidly spreading all over the world (3, 4). COVID-19 infection manifests with fever, headache, dry cough, fatigue, dysgeusia, anosmia and breath shortness, which can progress to acute respiratory distress syndrome due to severe bilateral pneumonia. A certain proportion of patients, in particular children, are asymptomatic (5, 6). Recently, skin manifestations have been described in COVID-19 patients (7-9). In addition to vesicular or maculo-papular skin rashes, livedoid/necrotic lesions and urticaria, chilblains-like lesions are increasingly reported in particular in adolescents and young adults (8-22). We also observed from last March an unusual number of patients, mainly teenagers, affected with chilblains-like lesions. Thus, we decided to investigate the aetiology of these lesions and possible relationship with COVID-19 infection in our patients.

We report the clinical, capillaroscopic, histopathological, ultrastructural, and laboratory findings in a prospective pediatric series of patients presenting with chilblains-like lesions during the COVID-19 pandemic.

MATERIALS AND METHODS

Study design and population. This study has been designed as a monocenter prospective study of pediatric patients presenting with chilblains-like lesions. Patients were screened and recruited in the second half of April at the Dermatological Unit of Bambino Gesù Children's Hospital, Rome, Italy.

Consecutive patients, with a clinical diagnosis of chilblain-like lesions established by a dermatologist, and giving specific informed consent were included in the study. Exclusion criteria were (i) patients aged less than 6 years and (ii) patients affected with a diagnosed autoimmune disease, in addition to (iii) patients or their legal guardians who refused to give the consent to participate to the study. The study was approved by our Institutional Ethical Committee and conducted in accordance with the Declaration of Helsinki.

Clinical evaluation. A standardized case-report form was created. Information on patient demographics, past and recent family and personal history, clinical features (lesion localization and type), symptoms, and follow-up was collected. In addition, results of different investigations performed (as detailed below) were recorded. Finally, clinical pictures were taken in all patients.

Study procedures. Patients recruited in the study underwent: (i) testing for SARS-CoV-2 on nasopharyngeal swab and, in selected cases, skin biopsy, (ii) digital videocapillaroscopy of finger- and toe-nailfolds, (iii) blood testing including serology for SARS-CoV-2, and (iv) lesional skin biopsy for histopathology, direct immunofluorescence, and, in 4 patients, transmission electron microscopy examination.

SARS-CoV-2 testing. "STARMag 96 X 4 Universal Cartridge kit" (Seegene Inc., South Korea), was used for extraction and isolation of nucleic acids from nasopharyngeal swabs and biopsy samples, followed by multiplex real-time reverse transcriptase (RT)-PCR assay for simultaneous detection of 3 target genes of SARS-CoV-2, RdRP and N genes specific for SARS-CoV-2, and E gene for all of Sarbecovirus including SARS-CoV-2 ("Allplex™ 2019-nCoV Assay", Seegene Inc.). In addition, serology for SARS-CoV-2 was performed using a chemiluminescent microparticle immunoassay (CMIA) for detection of IgG antibodies directed against the nucleocapsid protein of SARS-CoV-2 (Abbott Laboratories, USA). In parallel, all sera were tested using an anti-SARS-CoV-2 ELISA (Euroimmun, Germany) for the detection of IgG and IgA antibodies against the S1 domain of the spike protein including the immunologically relevant receptor binding domain.

Digital videocapillaroscopy. It was performed on all recruited patients with VideoCAP® 3.0 (DS Medica, s.r.l., Italy), according to standard procedures (23). Images were collected at 100X and 200X magnification. The following characteristics were evaluated: capillary density and architecture, pericapillary oedema, capillary morphology, capillary dimension (width of the apical limb of the capillary) and presence of avascular areas and microhemorrhages (24). Capillaroscopy images were independently reviewed by 3 dermatologists.

Blood testing. All patients underwent the following investigations: real-time PCR for Parvovirus B19, complete blood cell count, erythrocyte sedimentation rate, liver enzymes, creatinine, cryoglobulins, ANCA, antinuclear, anti-dsDNA, antiphospholipid and anticardiolipin, ENA and ribonucleic protein antibodies, C3, C4, immunoglobulins (IgG, IgM, IgA), and coagulation screening (D-dimers, prothrombin time, activated partial thromboplastin time, fibrinogen).

Histopathology, immunohistochemistry and direct immunofluorescence. Hematoxylin-eosin–stained sections of formalin-fixed paraffin-embedded tissue were reviewed under light microscopy. Three pathologists analyzed all cases independently. Histopathological features assessed in all cases included: apoptotic keratinocytes, spongiosis, exocytosis, vacuolar changes in the basal layer, inflammatory infiltrate (cell type, intensity and location), papillary dermal edema, vascular damage, and dermal fibrin and mucin deposition. The intensity of the lymphocytic infiltrate was graded 1-3, according to previous studies (25). Immunostains for CD3 (polyclonal, Dako Agilent), CD20 (clone L26, Dako Agilent), CD123 (clone BR4MS, Leica Novocastra), and Alcian blue stain were performed according to standard methods. Direct immunofluorescence (DIF) was performed using fluorescein isothiocyanate-conjugated anti-human IgG, IgM, IgA, fibrinogen, and complement fractions, C1q, C3c, and C4c (all from Dako).

Transmission electron microscopy (TEM). Skin biopsy specimens were fixed in 2% glutaraldehyde, post-fixed in 1% osmium tetroxide, dehydrated in graded alcohols, embedded in Epon resin, and sectioned on an ultramicrotome (Ultracut S, Reichert-Jung, Austria). Ultrathin sections were stained with uranyl acetate and lead citrate and observed with a transmission electron microscope (JEM-1400, Jeol, Japan).

RESULTS

Clinical features. The parents of nineteen out of twenty-one patients screened for chilblain-like lesions signed informed consent agreeing to the participation of their child in the study. Patient demographics, history and clinical findings are listed in Table 1. All patients were Italian and lived in Lazio Region (Central Italy). There was a predominance of male patients (M:F = 2.8:1), the mean age was 14.0 years (range, 11-17). Two patients were siblings. Patient history was negative for perniosis, autoimmune, neoplastic or coagulation disorders in all cases. Five patients (26%) reported fever and cough 1 to 2 months prior to development of skin lesions, and one patient presented diarrhea one week after skin manifestation onset. In addition, two patients recently stayed in areas with high prevalence of COVID-19 infection or hosted guests from these areas. In

nine cases (47%), family history was positive for fever, headache, cough, or gastrointestinal symptoms in one or more family members, 1 to 2 months prior to skin lesion onset. All patients were in good general health at the time of the first consultation and they had respected home confinement since March 9th, according to the Italian COVID-19 pandemic regulations. Interestingly, 16 patients (84%) reported barefoot walking during confinement (5 with socks). Skin lesions had appeared 12 to 40 days (mean, 22.2 days) before screening visit, presenting at first as toe swelling and erythema. On physical examination, lesions were localized exclusively to feet in all cases, they were bilateral and predominantly distributed to the distal toes, followed by heels and lateral foot margin, and rarely spread to the soles (Fig. 1, 2). All patients had toe swelling and erythema (Fig. 1a, b, d, Fig. 2a), accompanied by erythemato-violaceous roundish macules and purpuric lesions in 12 cases (63%) (Fig. 1c, Fig. 2b, c, d). In addition, some patients presented also pustules (Fig. 2a), and erosions covered by crusts (Fig. 1d, Fig. 2a, c). Eleven patients (57.8%) reported pain and/or itching concomitant to lesion onset and resolving spontaneously within 7-10 days. No one required analgesic or specific therapy. At follow-up (14 days after screening), patients still presented asymptomatic minimal erythema, swelling, brownish macules and/or crusts.

Digital videocapillaroscopy findings. Capillaroscopy was performed on both finger and toe nailfolds (Table 2). On fingers, the capillary density and architecture were normal in all patients. Pericapillary oedema was present in 11 out of 19 cases (Fig. 3a, c). Capillary dimension was abnormal (>20 μm) in 7 patients (Fig. 3c). Abnormal capillary shape was the most frequent feature (14 cases, 73.7%) (Fig. 3c). Microhemorrhages were detected in 5 patients (Fig. 3a). Given the particular anatomical nailfold conformation on feet, the first and second toes were mainly examined. Capillary density was normal in almost all analysed toes. Pericapillary oedema was less frequently detectable in toes than in fingers (5 versus 11 patients) (Fig. 3b, d), while toenail capillary dilation was present in 12 patients as observed in fingers (Fig. 3d). About half of the patients showed abnormal morphology of capillaries (Fig. 3b, d). The most striking capillaroscopic sign on toes were microhemorrhages, detected in 7 out of 19 patients (Fig. 3d).

Histopathology, direct immunofluorescence and ultrastructural findings. Results from histopathological analysis of skin biopsies obtained from 18 patients are summarized in Table 3.

Oedema of the papillary dermis was observed in 12 cases, being more prominent in 3 (Fig. 4c). Red cell extravasation was present in almost all patients (15, 88.2%). In all cases, a mild (12/18 cases) to moderate/intense (6 cases) perivascular lymphocytic infiltrate was seen in the superficial

and deep dermis where it also localized around the eccrine glands (Fig. 4a-e). A perivascular infiltrate was detected also in the hypodermis in all biopsies containing subcutaneous tissue (11/11) (Fig. 4a, e). Small vessels showed swollen endothelia in 15 cases. Intriguingly, signs of lymphocytic vasculitis with a prominent lymphocytic infiltrate and isolated neutrophils were observed in 3 cases, with involvement of a deep arteriole (Fig. 4b) and a venule in one case each. Fibrin thrombi were found in 2 patients (Fig. 4d). There was no evidence of fibrinoid necrosis in the vascular walls. Epidermis showed mild changes characterized by spongiosis (13 cases), slight vacuolation of basal layer (14 cases) (Fig. 4c), with lymphocyte exocytosis in 6 cases, and isolated apoptotic keratinocytes in 4. Alcian stain highlighted the presence of minimal mucin deposits mostly in the deep dermis in 10 cases, while in 7 it was more prominent with a diffuse involvement of subcutis (Fig. 4f). Immunostains showed a uniform phenotype in all cases, with a predominance of T-lymphocytes, CD3 positive (more than 95%), associated with scattered CD123 positive macrophages. Direct immunofluorescence performed on biopsies from 11 patients showed focal granular deposition of C3 in the walls of a few isolated dermal vessels (9 cases) (Fig. 5a, b). In addition, colloid bodies along the dermal-epidermal junction stained positive for IgM (4 cases) (Fig. 5c) and IgG (2 cases). TEM examination of lesional skin showed similar findings in the four patients examined. Several extravasated erythrocytes were present in the papillary dermis, which appeared diffusely oedematous (Fig. 6a). A dermal infiltrate chiefly composed of small to medium size lymphocytes with a few admixed macrophages was observed in a perivascular location but also scattered throughout the dermis (Fig. 6b). Dermal vessels frequently showed protruding endothelial cells with prominent intraluminal nuclei. In addition, some endothelial cells presented degenerative changes, with nuclear and cytoplasmic condensation and areas of denuded or interrupted basement membrane were observed (Fig. 6c, d).

SARS-CoV-2 testing results. Real-time PCR for SARS-CoV-2 on nasopharyngeal swabs from 19 patients and on skin biopsies from 3 patients proved always negative. In a similar way, serology for IgG antibodies directed against the nucleocapsid protein of SARS-CoV-2 was negative in all patients. Of note, serology for the detection of IgG and IgA antibodies against the S1 domain of the spike protein revealed: (i) positive IgG in one patient (n. 7 of Table 1), and borderline in 3 patients (n. 10, 13 and 14), (ii) positive IgA in 6 patients (n. 5, 9, 12, 13, 14, and 16), and borderline in 3 patients (n. 8, 10, and 19). Finally, all other blood tests performed were within normal limits in all patients.

DISCUSSION

Chilblain-like lesions are being increasingly reported in different European and non-European countries during COVID-19 pandemic (8-22). Indeed, first case series on skin manifestations during COVID-19 outbreak indicate that acral lesions are quite frequent: they were present in 106 of 277 dermatological outpatients (38.3%) in a French retrospective study (22) and in 79 of 375 cases (21%) in a Spanish prospective survey (9). Therefore, we decided to undertake a systematic study of clinical, capillaroscopic, histopathological, and SARS-CoV-2 infection in paediatric patients referred to us for this peculiar skin manifestation, in order to investigate a possible relationship with COVID-19 infection.

We recruited 19 consecutive cases during a two-week recruitment period in the second half of April. Interestingly, all our patients were adolescents (mean age: 14 years). Most previously published case series comprised both paediatric and adult patients making difficult a direct comparison (9, 14, 15, 17). Nevertheless, these studies reported a patient mean age ranging from 16 to 32.5 years. In addition, the first Italian case series included 63 patients, all adolescents, and a Spanish study on 27 paediatric patients reported a mean age of 14.44 years (12, 19). Altogether, these data indicate a prevalence of this peculiar manifestation in teenagers. In our series, males were more numerous than females (2.8:1), which was not confirmed in other case series showing about 1:1 sex ratio (12, 14, 15, 19, 22) and female predominance in a single study (9). In our patients, lesions were exclusively localized to feet, which represents the most frequently affected site also in previous reports, with 108 of 132 (81.8%), 37 of 41 (90%), 21 of 27 (78%), and 58 of 63 cases (92.7%) in different series (12, 14, 15, 19). In a minority of patients, hands alone were affected. Interestingly, 9 of our patients also had lesions on soles and heels, in addition to toes. Similar to previous reports, clinical manifestations in our patients comprised swelling, erythema, purpuric macules and papules, pustules and crusts. In our cases, cutaneous manifestations lasted more than one month and no relapses were observed. Although shorter disease duration has been reported in some cases series (9, 15), all reports agree on spontaneous resolution without sequelae. Overall, the demographic (similar sex ratio), epidemiological (onset with cool to warm temperatures and recent great increase in incidence) and clinical (involvement of soles and heels) of chilblain-like lesions during the COVID pandemic distinguish them from idiopathic perniosis. Furthermore, in keeping with literature data, laboratory findings, including autoimmunity and

coagulation screening, were negative or within norm in our patients, excluding connective tissue diseases.

We performed for the first time a videocapillaroscopy characterization of chilblain-like lesions showing the presence of pericapillary oedema, ectatic and abnormal capillaries, and microhemorrhages in a significant proportion of cases. Of note, our patients presented anomalies both on hands and feet, even though skin lesions were limited to feet in all cases. Thus, this finding suggests that chilblain-like lesions represent manifestations of a systemic process. Although limited data are available about the physiopathologic morphology of toe capillaries, the values of the major capillaroscopic parameters, such as capillary size and density do not differ significantly from fingers (26). In our series, the same percentage of patients (36.8%) presented dilated capillaries on hands and feet, while pericapillary oedema and microhemorrhages were more common in toe than finger nailfolds (14 versus 8 and 7 versus 4 cases, respectively). Altogether, these features appear more severe than those described in idiopathic pernio where microhemorrhages were not detected (27). Indeed, our capillaroscopy findings well match the clinical features of erythema, oedema, and purpuric lesions and the histological ones of dilated capillaries, superficial dermal oedema, with extravasated red blood cells observed in our patients. Histopathological examination showed a perivascular, superficial and deep, T-lymphocytic infiltrate and a combination of other findings considered more characteristic of either idiopathic chilblains or chilblain lupus erythematosus. The morphologic overlap between these two entities has been extensively investigated (25, 28, 29). Interface dermatitis with basal layer vacuolation and necrotic keratinocytes together with a perivascular dermal infiltrate without perieccrine distribution have been considered markers of chilblain lupus. By contrast, perieccrine inflammatory infiltrate and papillary dermis oedema/spongiosis are frequent findings in chilblains (25, 28). Our cases showed hybrid features: (i) a perieccrine infiltrate in all biopsies, and papillary oedema/spongiosis in about 70% of the cases, more in keeping with chilblains, (ii) basal membrane smudging and mucin deposition, both detected in the large majority of the cases, are more reminiscent of lupus (25, 27, 28). Very few chilblain-like lesions with potential COVID-19 infection association have been histologically investigated to date (18, 20-22). Similar to our findings, a lymphocytic perivascular and periadnexal infiltrate, basal layer vacuolation and, in 3 biopsies, microthrombi were observed (18, 20, 22).

Despite increasing reports on chilblain-like lesions during COVID-19 pandemic, the possible relationship between this skin manifestation and SARS-CoV-2 infection remains matter of debate.

All our patients were in good general health at the time of first examination and, except for an episode of diarrhea in a single patient, did not develop any extracutaneous symptom during the follow-up period. However, the majority of them and/or their cohabitants (11/19, 58%) reported flu-like symptoms one to two months prior to skin lesion onset. Previous reports varied widely as to the presence of suspected and/or confirmed symptomatic COVID-19 infection in patients with chilblain-like lesions. Galván Casas et al. reported chilblain-like lesions in 71 patients with either confirmed COVID-19 infection (41%) or suggestive symptoms (59%) (9). However, the percentage of patients with extracutaneous symptoms was much lower in other series ranging from 0 % to 44.4% (14, 15, 17, 19, 22). To date, a limited number of patients underwent SARS-CoV-2 testing (9, 11-22). Interestingly in 3 reported patients with confirmed COVID-19 infection, cutaneous manifestations started 17 to 28 days after COVID-19 symptoms (21), indicating that chilblain-like lesions can appear late in disease course, as also suggested by our patient history. SARS-Cov2 testing by real-time RT-PCR on nasopharyngeal swabs and, in three cases, on skin biopsy proved negative in all our patients. However, the virus becomes undetectable in the upper respiratory airways usually after 6 to 11 days (30). Indirectly, the infection can be measured by detecting SARS-COV-2 specific antibodies in the serum (31). SARS-COV-2 serology has been performed to date in very few patients with chilblain-like lesions and found positive in 2 patients with positive swab (12, 19). IgG and IgA antibodies are secreted in patients with COVID-19 (32). Most of the IgA is localized at mucosal sites for protection against pathogens and control of the microbiota. IgA is produced in response to respiratory infections in the nasal and bronchial-associated lymphoid tissue (33). Besides its protective role, it has recently been demonstrated that IgA may also trigger inflammation at mucosal and non-mucosal sites (34). However, many questions are still open regarding serology in COVID-19 and most of the published data is on adults (31). The tests current available vary because of the different techniques used and the antigen against which the specificity is measured (34). We used two different methods: the first measures IgG antibodies against the SARS-COV-2 nucleoprotein (Abbott), and was negative in all patients, whereas the second (Euroimmun) detects IgG and IgA specific for the S1 domain of the spike protein (35). Importantly, 6 patients (31.6%) were positive for IgA, and 3 (16%) borderline. The observation that children have serum antibodies against SARS-COV-2 of IgA isotype suggests that their immune response is based on strong mucosal protection that might even impair the triggering of an IgG response.

In conclusion, our study shows that chilblain-like lesions during COVID pandemic have several epidemiologic, clinical, capillaroscopic and histological characteristics different from idiopathic chilblains, making high unlikely that patients are affected with idiopathic perniosis. Though we could not formally prove SARS-CoV-2 infection in our patients, history data and presence of anti-SARS-COV-2 IgA strongly suggest a relationship between skin lesions and COVID-19. Importantly, most patients developing chilblain-like lesions have mild COVID-19 disease or are completely asymptomatic, and no fatal cases have been reported to date, suggesting that chilblain-like manifestations predominantly occur in COVID patients with a favorable disease course. Finally, our findings highlight the need for further investigations on the mechanisms of SARS-CoV-2 infection in children and pathogenesis of chilblain-like lesions.

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FIGURE LEGENDS

Figure 1. Clinical features. Erythema and swelling of right foot toes (a); erythema and swelling, particularly marked on the fourth right toe (b); purpuric macules, more evident on the second toe of both feet (c); erythema, mild swelling and multiple crusts on left toes (d).

Figure 2. Clinical features. Diffuse swelling, erythema, and crusts on the left foot, and two pustules (arrows) on the big toes (a); purpuric macules on the lateral aspect of the right heel (b); confluent purpuric macules mostly covered by crusts on both heels and posterior ankles (c); brownish purpuric macules on the soles (d).

Figure 3. **Videocapillaroscopy findings**. Multiple and synchronous microhemorrhages (asterisks), pericapillary oedema (arrows) on the finger nailfold (a); abnormal morphology of the capillaries (white circle) and pericapillary oedema (arrows) on the toe nailfold of the same patient (b). Pericapillary oedema (arrows), dilated capillaries (white triangle), and abnormal capillary morphology (white circle) on the finger nailfold (c); numerous and prominent microhemorrhages (asterisks), marked abnormal capillary morphology (white circles), and pericapillary oedema (arrows) on the toe nailfold of the same patient (d).

Figure 4. Histopathological findings. Representative low-power magnification of a punch skin biopsy showing perivascular inflammatory infiltrate in the superficial and deep dermis and subcutaneous tissue (a); higher magnification of the arteriole indicated with an arrow in (a) shows an intramural lymphocytic infiltrate (b); a scale-crust, epidermal spongiosis, basal layer smudging (arrow) and oedema of papillary dermis with extravasated erythrocytes are evident in (c); a dermal capillary blood vessel with intraluminal thrombus is visible in (d); dense perieccrine and perineural dermal inflammatory infiltrate associated with mucin deposits (e) highlighted by Alcian blue staining shown in (f). a-e: haematoxylin-eosin staining; bars: 500 μm in a; 50 μm in b and d; 100 μm in c, e and f.

Figure 5. Direct immunofluorescence findings. Granular deposits of C3 in the wall of vessels in the papillary and deep dermis (a and b, respectively) and focal deposits along the dermal-epidermal junction; colloid bodies at the dermal-epidermal junction staining positive for IgM (c). Bars: 50 μm in a and b; 100 μm in c.

Figure 6. Ultrastructural features. Extravasated red blood cells (R) are visible in an oedematous (asterisks) papillary dermis (a, "F" denotes two fibroblasts); a dermal infiltrate chiefly composed of small to medium size lymphocytes (L) is observed in the papillary dermis (b); protruding endothelial cells with prominent intraluminal nuclei, one with condensed chromatin (asterisk in c), line two dermal vessels (c and d), one showing partly denuded and interrupted basement membrane (asterisk in d). Bars: 10 μm in (a) and (b), 5 μm in (c) and (d).

Table 1. Demographics, history, clinical features in 19 patients with chilblain-like lesions

Pt.	Age (yrs)	Sex	Recent family history°	Recent personal history°	Lesion localization	Lesion type	Symptoms
1	13	F	Parents and 2 siblings: headache, fever, abdominal pain, one month before	Fever, headache, sore throat, one month before	Toes	Erythema, swelling*	Intense pain
2	13	F	Negative	Negative	Toes	Erythema, swelling, purpuric macules, crusts	Intense pain
3	12	M	Parents: sore throat and fever, two months before	Fever, two months before	Toes, heels	Erythema, swelling, pupuric macules	Intense itching
4	15	M	Mother: cough and fatigue, one month before	Negative	Toes, heels, soles	Erythema, swelling, violaceous macules	No
5	14	M	Negative	Negative	Toes	Erythema, swelling	Itching
6	14	M	Negative	Negative	Toes, heels, soles	Erythema, purpuric macules, papules, crusts	Itching
7	13	F	Negative	Negative	Toes	Erythema, swelling, purpuric macules, crusts	Intense icthing
8	15	M	Grandfather** fever and cough, one month before	Negative; travel to Milan, 1.5 month before	Toes, heel, soles	Erythema, swelling, purpuric macules, pustules, crusts	Pain, itching
9	17	M	Negative	Negative, hosting a friend from Northern Italy, 2 months before	Toes, heels, soles	Erythema, crusts	Pain, itching
10[#]	13	M	Negative	sore throat, fever, diarrhea, 1.5 month before	Toes, heels	Erythema, purpuric macules, pustule	Burning, itching
11[#]	16	M	Brother: sore throat, fever, diarrhea, 1.5 month before	Negative	Toes	Erythema, swelling	Asymptomatic
12	15	M	Negative	Negative	Toes, heels, soles	Erythema, swelling, purpuric macules, crusts	Asymptomatic
13	17	M	Negative	Negative	Toes, heels, soles	Erythema, swelling, pupuric macules, pustule, crusts	Asymptomatic
14	17	M	Father and his partner: long-	Negative	Toes, heels,	Erythema, swelling,	Asymptomatic

			lasting fever and cough, one month before		soles	purpuric macules, crusts	
15	12	F	Father: fever and cough started 1.5 month before	Negative	Toes	Erythema, swelling, purpuric macules, crusts	Intense pain
16	12	M	Negative	Negative	Toes	Erythema, swelling	Asymptomatic
17	11	F	Negative	Fever, 2 months before	Toes	Swelling, purpuric macules, crusts	Asymptomatic
18	14	M	Negative	Diarrhea one week after skin lesion onset	Toes	Erythema, swelling, crusts	Burning
19	14	M	Negative	Fever and cough, two months before	Toes, heel, soles	Erythema, swelling, purpuric macules, crusts	Asymptomatic

°Family and personal history timing are referred to the onset of skin manifestation; *Swelling was limited to toes in all patients; ** Patient cohabitant; #Patients 10 and 11 are brothers

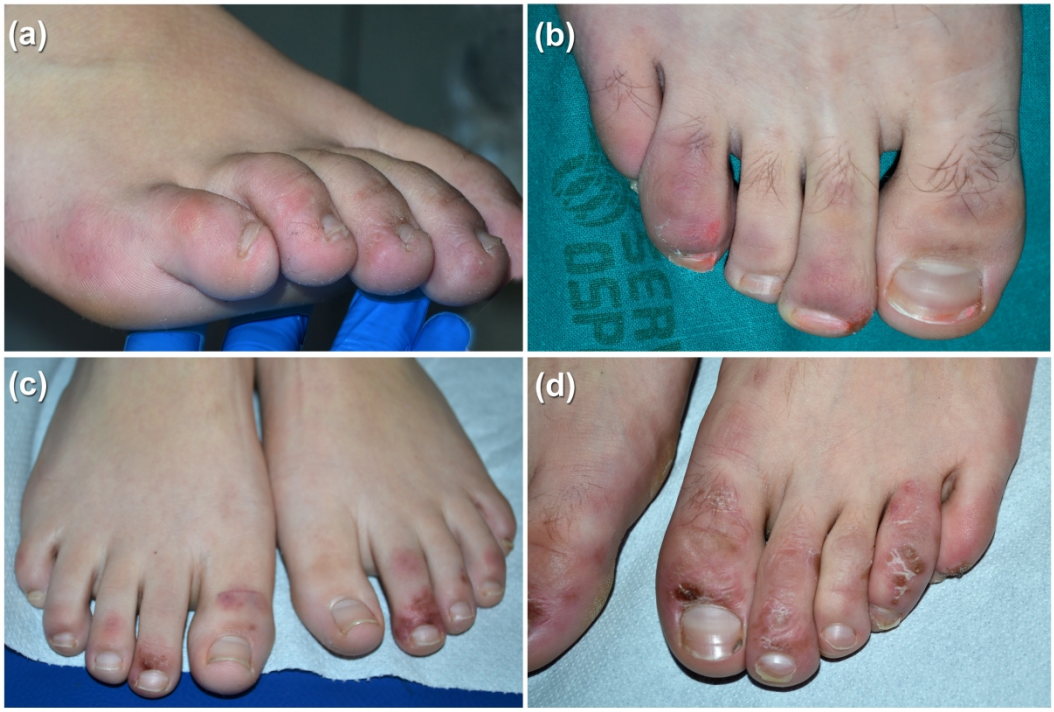
Table 2: Capillaroscopic features in 19 patients with chilblain-like lesions

Capillaroscopic characteristics		Patient number (%)	
		Fingers	**Toes**
Capillary density	Normal	19 (100%)	18 (94.7%)
	Reduced (<7 capillaries/mm)	0 (0%)	1 (5.3%)
Pericapillary oedema	Absent	8 (42.1%)	14 (73.7%)
	Present	11 (57.9%)	5 (26.3%)
Capillary dimension	Normal (<20 μm)	12 (63.2%)	12 (63.2%)
	Abnormal (>20 μm)	7 (36.8%)	7 (36.8%)
Morphology	Normal	5 (26.3%)	9 (47.4%)
	Abnormal	14 (73.7%)	10 (52.6%)
Microhemorrhages	Absent	15 (79%)	12 (63.2%)

Table 3. Pathological features in 19 patients with chilblain-like lesions

Pathological features		**Patient N* (%)**
Papillary dermis edema		12/18 (67%)
Extravasated erythrocytes		15/18 (83%)
Perivascular and perieccrine lymphocytic infiltrate	mild	12/18 (67%)
	moderate/marked	6/18 (33%)
	involving subcutis	11/11 (100%)
Lymphocytic vasculitis		3/18 (17%)
Fibrin thrombi		2/18 (11%)
Spongiosis		13/18 (72%)
Basal epidermal layer vacuolation		14/18 (78%)
Exocytosis		6/18 (33%)
Apoptotic keratinocytes		4/18 (22%)
Mucin	minimal	10/18 (55%)
	prominent	7/18 (39%)

*N: number



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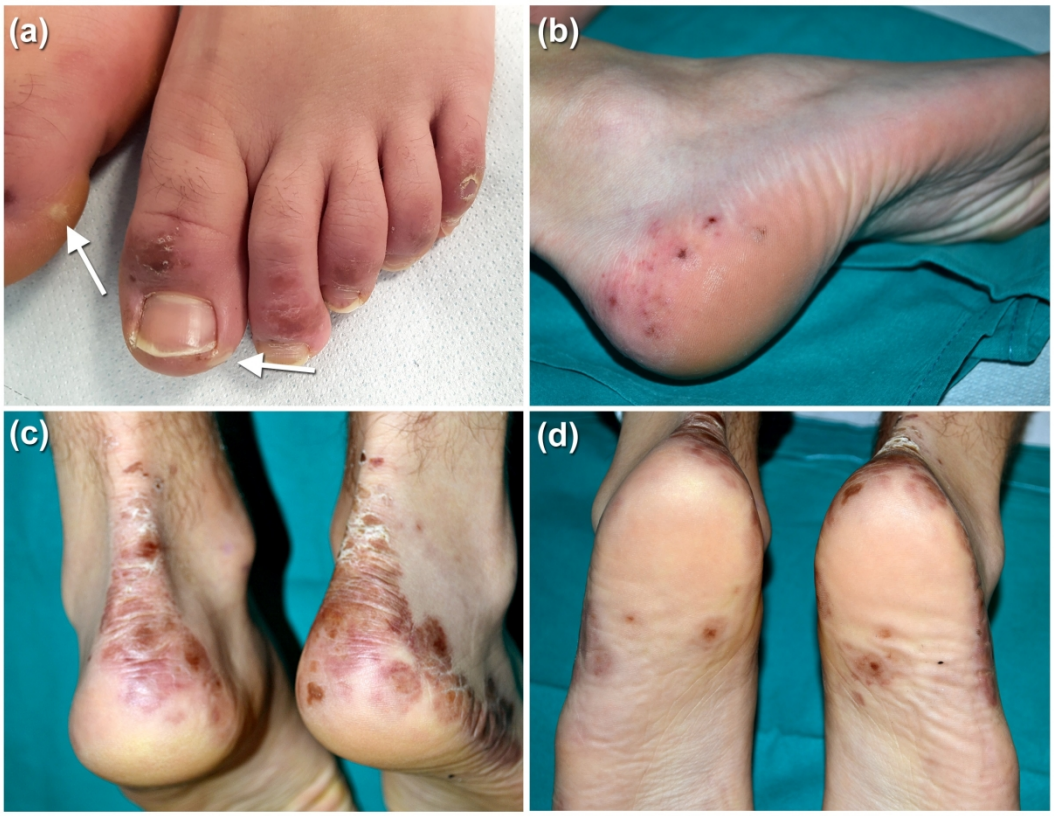

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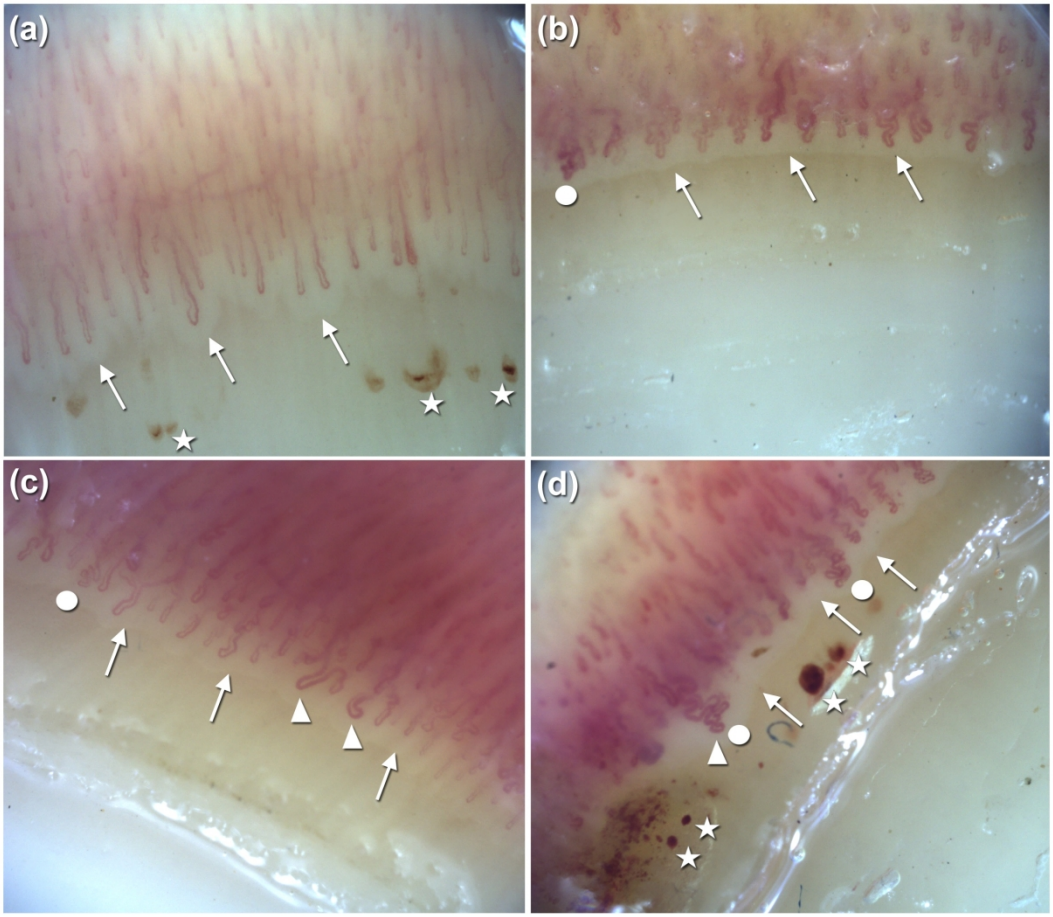

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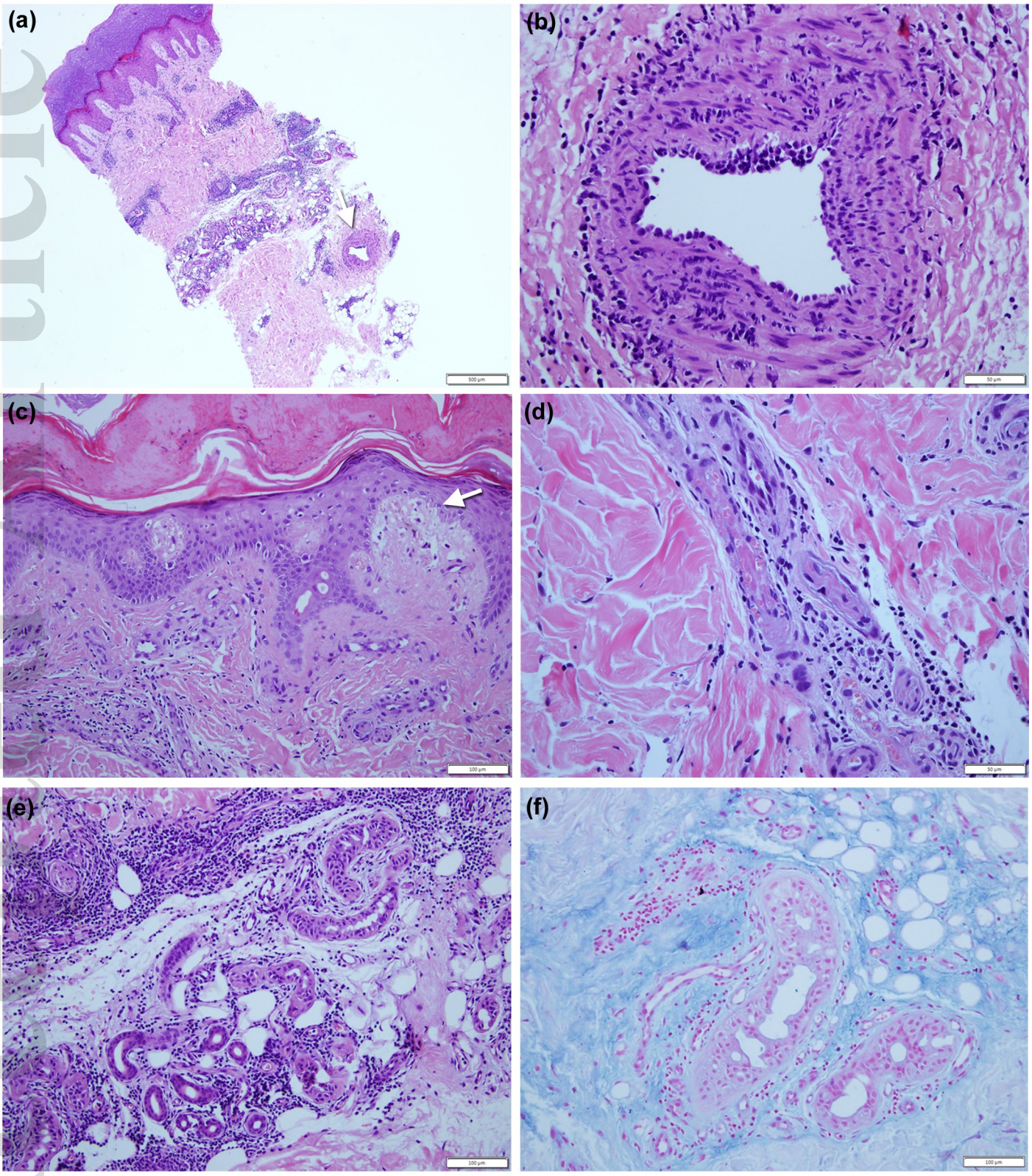

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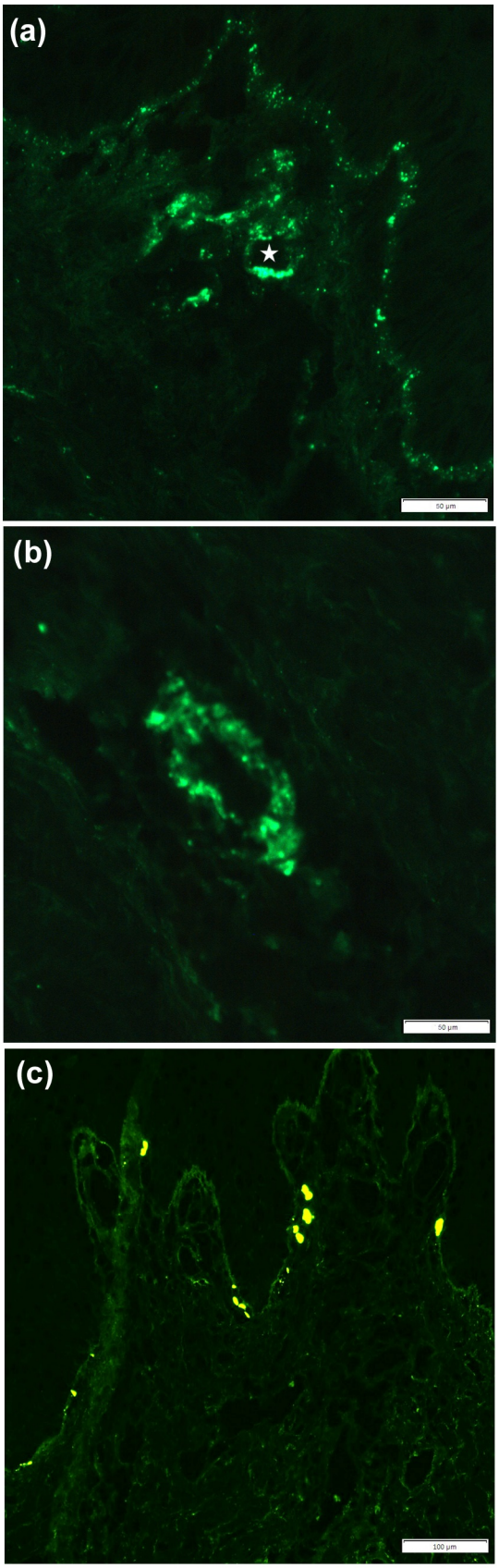

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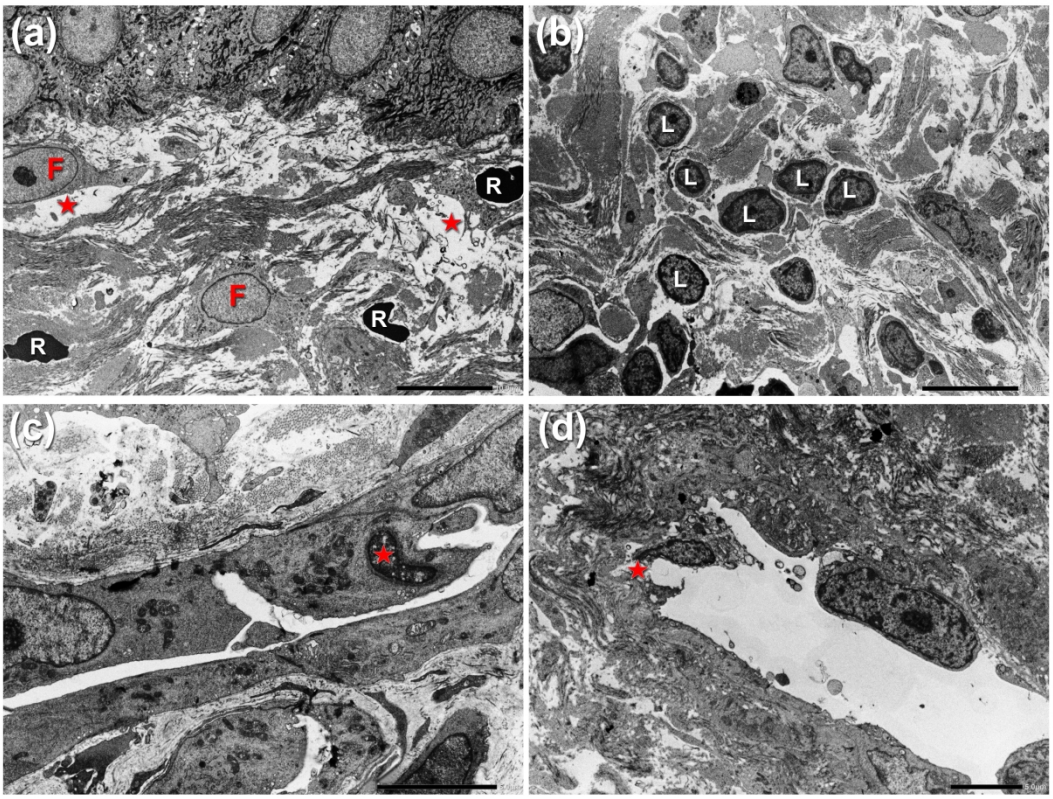

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